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Weight loss in patients with hematological neoplasias is associated with immune system stimulation

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Summary. Weight loss is the main symptom of so-called tumor cachexia. The pathogenetic mechanisms underlying cachexia are poorly understood; however, it appears that enhanced formation of cytokines such as interferon-γ and tumor necrosis factor-α are involved. In 94 patients suffering from hematological neoplasias we compared body weight changes with serum neopterin, tryptophan, and kynurenine. Biochemical changes, the formation of neopterin, the degradation of tryptophan are closely related to interferon-γ activity. The majority of our patients had increased neopterin and decreased tryptophan concentrations. Weight loss was seen particularly in patients with higher neopterin and lower tryptophan values. An association between higher neopterin levels and greater weight loss was apparent at study entry and during the follow-up of patients. Our data support the concept that weight loss is closely linked to endogenous interferon-γ activity.

Key words: Cachexia – Hematological neoplasia – Cytokines – Neopterin – Tryptophan metabolism

Weight-loss is the main symptom and in most cases the first sign of so-called tumour cachexia. The frequency of weight loss varies from one tumor type to another [18]; about one-third (36%) of breast cancer patients present with weight loss whereas in patients with cancer of the pancreas the proportion is between 70% and 83% [8]. Some 30–50% of patients with malignant lymphomas experience weight loss before diagnosis is established [3]. In addition to fever and night sweating, a decrease of 10% or more in body weight within the past 6 months is judged as a "B symptom" which indicates poor prognosis [19].

The pathogenetic mechanisms underlying weight loss and cachexia are poorly understood. Soluble factors such as tumor necrosis factor-α (TNF-α) [19], interleukin-1 [17], interferon-γ (IFN-γ) [15], and interleukin-2 [3] appear to be involved in the development of tumor cachexia. A variety of cytokines, including TNF [8] and interleukin-6 [14], and growth factors such as granulocyte colony-stimulating factor [13, 16] are released by activated lymphocytes and monocytes/macrophages, which play a crucial role in the complex network of cytokines. However, direct information on cytokine profiles is often incomplete because in vivo measurement is complicated as these substances do not necessarily remain in the circulation but bind rather to target cells or to serum soluble forms of their receptors.

This study addressed the question of whether weight loss is associated with immune activation in patients with hematological disorders. To quantify immune activation we determined serum concentrations of neopterin, tryptophan, and the tryptophan degradation product kynurenine. In vitro and in vivo, neopterin formation and tryptophan degradation are induced by stimulation of cellular immunity [7, 12].

Patients and methods

Included in the study were 94 patients (53 males, 41 females) suffering from hematological disorders: 57 had non-Hodgkin's lymphoma (NHL; 9 stage I, 13 stage II, 9 stage III, 26 stage IV), 23 suffered from Hodgkin's disease (HD; 1 stage I, 9 stage II, 7 stage III, 6 stage IV), and 14 had multiple myeloma (MM; 3 stage I, 3 stage II, 7 stage-III), including one patient with monoclonal gammopathy of unknown significance (MGUS). In 34 patients (36%) evaluation was performed before

Abbreviations: NHL = non-Hodgkin's lymphoma; HD = Hodgkin's disease; MM = multiple myeloma; MGUS = monoclonal gammopathy of unknown significance; IFN-γ = interferon-γ; TNF-α = tumor necrosis factor-α

the start of treatment, 26 (28%) were under therapy, and 34 (36%) were without treatment because of remission or stable disease. Staging of patients with HD and NHL was performed according to the Ann Arbor classification [4]; patients with MM/MGUS were staged according to the classification of Durie and Salmon [9]. Body weight was examined at any visit and values prior to the study were taken from the medical records. The extent of weight loss within the previous 6 months was measured as absolute and proportional body weight.

Serum neopterin concentrations were determined by radioimmunoassay (Neopterin Immutest, Henning, Berlin, FRG) [20]. Serum levels of tryptophan and kynurenine were measured by high-pressure liquid chromatography on reversed phase carbon-18 material using on-line deproteinization of specimens as described elsewhere [23]. Tryptophan was detected by its native fluorescence (excitation, 285 nm wavelength; emission, 360 nm wavelength) and kynurenine by UV absorption at 360 nm wavelength [11]. As a measure of protein status in patients, serum albumin was determined routinely using a fully automatic bromocresol purpur method (Hitachi, Japan). Normal values of albumin are between 38 and 49 g/l. In a number of patients more than one simultaneous measurement of body weight and serum neopterin concentration was available and could be compared to the clinical course.

For the evaluation of differences between various groups of patients we used the Kruskal-Wallis rank sum test. Incidences were compared by χ^2 test. For the definition of strength and significance of correlations Spearman's rank correlation coefficient was used. Values below $P=0.05$ were considered statistically significant.

Results

Loss of body weight within the past 6 months was found in 29 patients (31%); average weight loss in these patients was 8.2 kg (11.2%) of body weight. Weight loss was seen in 3 of 23 (13%) patients with HD, in 22 of 57 (39%) with NHL, and 4 of 14 (29%) with MM/MGUS. Weight loss was more frequent in patients who were examined before the start of therapy (18 of 34, 53%) or during therapy for active disease (8 of 26, 31%) compared to patients with stable course of disease or remission (4 of 34, 12%; $\chi^2=13.29$; $P<0.001$). Likewise, average weight loss was higher in the former groups of patients (pretreatment, 8.7%; treatment, 10.9%) than in the latter (untreated, 5%).

Table 1. Neopterin, tryptophan, kynurenine, and albumin concentrations

	Neopterin (nmol/l)	Tryptophan (μmol/l)	Kynurenine (μmol/l)	Albumin (g/l)
HD	7.8±3.6	56.4±13.1	2.3±1.1	42.4±3.8
NHL	22.4±34.1	50.5±16.9	2.8±1.4	40.3±6.2
MM/MGUS	13.6±10.7	44.9±12.9	2.5±1.0	39.4±7.5
Normal range	≦8.7 [20]	≧65 [11]	≦3.5[11]	38–49[a]

[a] Normal range of laboratory controls

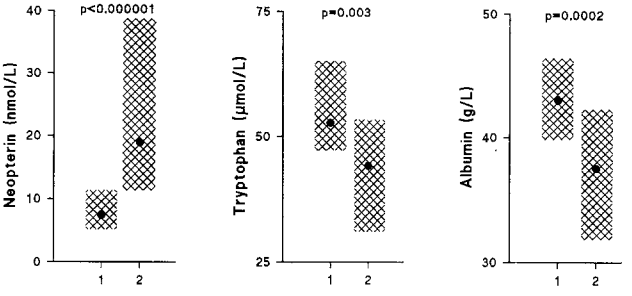

Fig. 1. Serum neopterin, tryptophan, and albumin concentrations (medians, 25th and 75th percentiles are shown) in patients with stable weight (*1*) and those with weight loss (*2*)

Compared to the normal range of healthy controls [20] neopterin levels were significantly raised in patients suffering from NHL and in those with MM/MGUS ($P<0.001$; Table 1); in contrast, serum tryptophan concentrations were significantly decreased ($P<0.001$). Serum kynurenine levels did not differ from normal values. Average serum albumin concentrations were within the normal range in all groups of patients, but the values were in the lower third of normal (Table 1).

Patients with weight loss showed significantly higher levels of neopterin than patients with stable weight ($H=31.82$; $P=1.7\times10^{-8}$; Fig. 1). There was a clear association between neopterin concentrations and the degree of weight loss ($rs=0.625$; $P<0.001$). Patients with weight loss had significantly lower tryptophan levels than those without (Fig. 1). There was an inverse correlation between tryptophan levels and the degree of weight loss ($rs=0.377$; $P=0.07$) whereas kynurenine was not different. In addition, we found a negative correlation between tryptophan and neopterin concentrations ($rs=-0.440$; $P<0.01$) and a positive correlation between neopterin and kynurenine ($rs=0.453$; $P<0.01$). The most significant correlation was found between the tryptophan/kynurenine ratio and the neopterin concentration ($rs=0.698$; $P<0.001$).

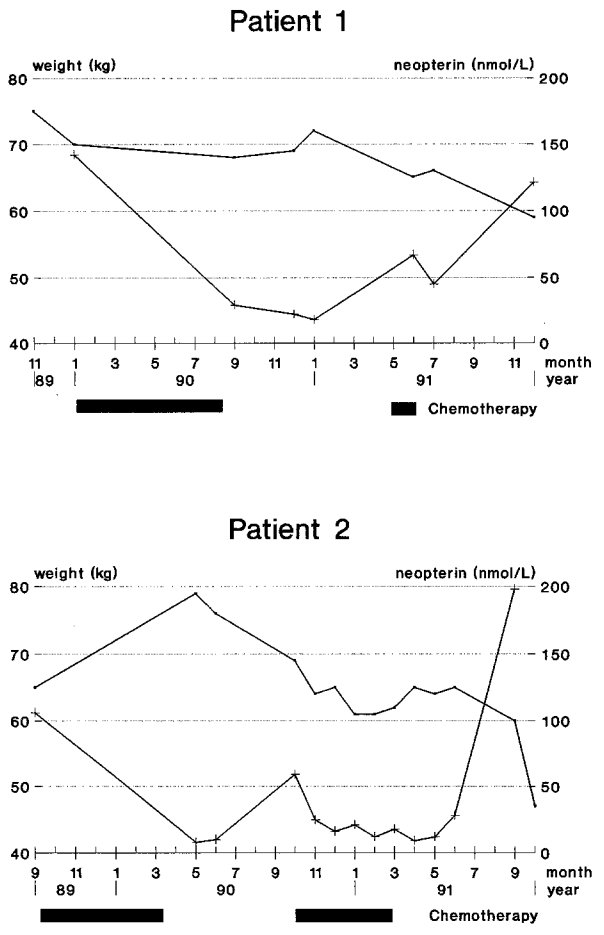

Fig. 2. Course of body weight (*dots, upper curves*) and neopterin concentrations (*crosses, lower curves*) in two patients with NHL over a 2-year period

Albumin levels of patients with weight loss were significantly lower than of those with stable weight. Similar to the changes in neopterin concentrations, the degree of hypalbuminemia was expressed more clearly in patients with marked weight loss (Fig. 1). There was a significant inverse correlation between albumin and neopterin concentration ($rs = -0.470$; $P < 0.0001$); the correlation was weaker to kynurenine ($rs = -0.277$; $P = 0.052$) and absent to tryptophan.

Similar associations between weight loss and immunologically induced changes were seen in data obtained during the follow-up of patients. Figure 2 presents representative courses of two patients, of whom serial neopterin measurements were performed at several time points during phases with and without chemotherapy. Increases in serum neopterin concentrations usually coincided with a drop in body weight and vice versa, but at certain time points weight loss was delayed (e.g., patient 2 in Fig. 2: a dramatic increase in neopterin was seen at time point 9/91; during the following month the patient lost 13 kg in weight).

Discussion

A significant percentage of our patients with malignant diseases showed signs of immune activation as detected by raised neopterin levels and significant degradation of tryptophan. Stimulation of cultured human monocytes/macrophages with IFN-γ induces the secretion of D-erythro-neopterin [10]. Among many biochemical changes, IFN-γ also stimulates indoleamine (2,3)-dioxygenase, which degrades tryptophan to form kynurenine in a variety of human cells including macrophages and malignant cell lines [22]. Both pathways mediated by IFN-γ are synergistically enhanced by TNF-α [24]. Measurement of neopterin, tryptophan, and its metabolites is also an indirect but sensitive way to detect and monitor cellular immune activation in patients. Formation of neopterin and degradation of tryptophan were observed in patients treated with cytokines [3, 21] and in patients suffering from diseases which are associated with activation of cellular immunity [11, 21]. Associations between endogenous IFN-γ, neopterin formation, and tryptophan degradation have been also found in infectious disorders such as human immunodeficiency virus infection [11] and Lyme's disease [12].

Low tryptophan levels in our patients were due to degradation. We observed significant positive correlations of neopterin with kynurenine and the kynurenine/tryptophan ratio. Kynurenine is the first metabolite of the tryptophan degradation pathway; therefore the kynurenine/tryptophan ratio represents the quotient of product versus substrate of indoleamine (2,3)-dioxygenase activity. Thus, the data indicate chronic stimulation of immune cells and particularly of monocytes/macrophages (as the primary source of neopterin) to take place in patients. There was no apparent increase in kynurenine levels, which is not surprising and agrees with in vitro data: kynurenine is rapidly further metabolized downstream in the tryptophan degradation pathway, and metabolites such as anthranilic acid and 3-hydroxyanthranilic acid are formed.

A significant correlation between serum IFN-γ and neopterin levels was described earlier in patients with hematological neoplasias [6]. In this study we found a clear association between weight loss and the quantitative changes in immune activation markers. The changes were essentially the

same in all subgroups of patients, HD patients behaving closest to normal. However, 8 out of the 23 patients with HD were in complete remission when examined.

Weight-loss in tumor patients is a sign of the complex syndrome of cachexia. Many metabolic changes have been described to be associated with cachexia, including disturbances in lipid, carbohydrate, and protein metabolism (for review see [18]). Soluble factors play a crucial role in mediating these changes. They are produced by the malignant cells themselves or by cells of the host as a reaction against the growing tumor. Our results indicate such a role for endogenous cytokines such as IFN-γ and TNF-α, particularly for stimulated macrophages in this network; the best correlations were found between the degree of weight loss and neopterin concentrations. While our findings are restricted to patients with hematological neoplasias, recent data of others indicate that the relationships found may be relevant for malignant diseases more generally. A cell line producing IFN-γ induced cachexia in an animal model [20], and, similarly, elevated levels of serum TNF and TNF mRNA were detected in peripheral blood mononuclear cells of patients with various types of cancer [1]. Both IFN-γ and TNF synergistically induce and enhance macrophage activation and neopterin secretion as well as tryptophan degradation.

In previous studies we demonstrated that higher neopterin levels are associated with a worse prognosis in patients with hematological neoplasias [5]. Additionally, we found an association between increased serum IFN-γ and neopterin concentrations and the development of so-called tumor anemia [6, 7].

In sum, there are a number of arguments favoring the hypothesis that chronic immune activation and endogenous release of cytokines play a role in the pathogenesis of symptoms in patients with hematological disorders, and that weight loss and cachexia are closely linked to endogenous IFN-γ and the activation status of macrophages. Although our data provide only indirect evidence as endogenous formation of cytokines was not directly assessed, there is some indication that reducing the degree of immune stimulation may be beneficial.

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